Analytical Method Development and Validation of Favipiravir by UV Spectrophotometric Method

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Abstract-Analytical chemistry is a branch of chemistry that deals with the separation, identification and determination of components in a sample. Aim of the present work was to develop a simple, rapid and economical method for estimation of Favipiravir by UV-Visible spectrophotometric method. Favipiravir is a modified pyrazine analogue that was initially approved for therapeutic use in resistant case of influenza. The linearity was studied by performing three independent analyte curves with six concentration levels ranging between (2-12µg/ml). The recovery results showed that the proposed method has an acceptable level of accuracy for Favipiravir which is from 50-150%. The %RSD values of precision was found to be < 2. LOD was found to be 0.0178 whereas LOQ was 0.054. The method was validated as per the guidelines laid by ICH. The results of validation test were found to be satisfactory and therefore this method can be applied successfully to analysed drug formulation.

INTRODUCTION

Pharmaceutical analysis comprises the procedures necessary to determine the "identity, strength, quality and purity" of compounds[1]. The pharmaceutical industry is one of the most regulated industries worldwide because the drugs produced must be safe and effective.[2]The Food and Drug Administration requires that raw materials are tested before manufacturing pharmaceutical products to establish their identity, purity, and Quality.[3]

Analytical chemistry is separated into two predominant classes, a qualitative evaluation that is to say the identification with regard to the chemical additives exists in the sample, whereas quantitative evaluation estimates the amount of positive detail or compound within the substance[4]. One of the earliest instrumental techniques for analysis is UV-Vis spectroscopy. The UV-Vis delivers details based on the degree of absorption or transmittance of a varied wavelength of beam light and the various responses of sample [5]. Analytical chemistry is a multifaceted tool with important applications in health and medicine. Although medical device technology is often associated with biomedical engineering and materials science, analytical chemistry plays an important and perhaps under-recognized role. In particular, the need to measure things is fundamental to the regulatory science of medical devices[6].

Spectroscopy is a branch of science that deals with the study of interaction of UV radiation with matter. Spectroscopy is a most useful tool available for study of atomic & molecular structure[7]. Development method is essential for discovery, development, and evaluation of medicines in the pharmaceutical formulation[8]. In recent years, spectroscopic techniques have come to be regarded as attractive and promising analytical tools for analyses conducted in research, control or industrial laboratories.[9]These techniques are increasingly considered by analysts as an obvious solution. This trend stems from instrumental developments, the extensive use of computers and the development of appropriate chemometric procedures.[10]

Favipiravir is an antiviral medication effective against all three types of influenza viruses (A, B, C). Favipiravir chemical name is 6-fluoro-3-hydroxy-2-pyrazine carboxamide, with the molecular formula $C_5H_4FN_3O_2$. This drug is a derivative of pyrazine carboxamide. This makes its versatile drug in the treatment of viral infections[11].

Favipiravir(T-705) is a synthetic prodrug, first discovered while assessing the antiviral activity of chemical agents active against the influenza virus. Within the tissue, the molecule undergoes phosphoribosylation to favipiravir-RTP, which is the active form of this drug. This molecule acts as a substrate for the RNA-dependent RNA polymerase (RdRp) enzyme, which is mistaken by the enzyme as a purine nucleotide, thus inhibiting its activity leading to termination of viral protein synthesis[12]. Favipiravir (T-705, 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) is a broad-spectrum antiviral agent inhibiting diverse kinds of viruses, such as arenaviruses, bunyaviruses, flaviviruses, foot-and-mouth disease virus and influenza viruses, in cell culture and in animal model systems.[13] The mode of action of the compound has been studied in influenza virus infections but not against other viruses. T-705 undergoes conversion into a nucleotide analogue, with monophosphate (T-705 RMP) and triphosphate (T-705 RTP) derivatives detected in cell lysates when using radioactive T-705 as a probe.[14]

MATERIALS AND METHOD

The Shimadzu prominence high performance liquid chromatography (HPLC) system was employed for the development and validation of a method to determine FVPR. This analytical instrument is equipped with a UV detector utilizing a deuterium lamp as the light source, a quaternary pump for precise solvent delivery, and an auto-injector for sample introduction. The chromatograms were analysed using lab solution software.

To ensure accurate pH conditions, the buffer solution was adjusted using an Eutech pH meter. Solvent degassing was carried out using an Ultrasonic sonicator bath, and filtration was performed using Axiva India membrane filters with a pore size of 0.45 micrometre nylon filters. All the chemicals and reagents used were of high purity and met analytical grade standards. For the pharmaceutical component of the study, PIRAVAFI tablets with batch number 2132642. Were collected. Gift sample of drug was obtained from Spectrum pharma research solutions Hyderabad.

PREPARATION OF SOLUTIONS

Involved systematically diluting the stock solution to achieve the desired concentrations for the UV spectroscopic method development and validation, ethanol was utilised as the diluents. Working standard solutions for uv spectroscopy were also prepared by diluting the stock solution based on the parameters under investigation, such as linearity, accuracy, recovery, limit of detection and limit of quantification (LOQ)[7].

Preparation of standard stock and working standard solutions

Accurately weighed 100mg of Favipiravir was transferred to a standard flask. Five ml of methanol was added, sonicated and then volume was made up to 100ml with water to give a solution 1mg/ml. This solution was labelled as standard stock solution. From the standard stock solution, 10ml was pipetted out into a 100ml volumetric flask and diluted upto mark with distilled water to yield a solution of strength 100µg/mL. This solution was labelled as working standard solution.

Determination of absorption maxima of favipiravir

From working standard solution, 1ml was pipetted out into a 100ml volumetric flask and diluted upto mark with distilled water to yield a solution of strength 10µg/mL. The spectrum of this solution was scanned over 200-400nm range in a UV spectrophotometer against distilled water as blank to estimate the absorption maxima (favipiravir).

METHOD DEVELOPMENT AND VALIDATION

According to ICH guidelines the proposed uv methods were suggested to confirm methods for validation.

Linearity

The linearity of analytical procedure is its ability to obtain test results directly proportional to concentration of analyte in samples[15]. The linearity was studied by performing 3 independent analytical curves with 6 concentrations levels ranging between 2-12µg/ml. Appropriate volume of aliquot from Favipiravir standard stock solution was transferred to volumetric flask of 10ml capacity. The volume was adjusted to mark with 0.1N HCL to produce standard solution.

Accuracy

Accuracy is the closeness of the test results obtained by the method of true value[16]. Solutions were prepared

in triplicate at levels 50%, 100% and 150% of test concentration using Favipiravir by working standard solution as per the test method and taen absorbance of each solution in triplicate values. The recovery resu,lts showed that the proposed method has an acceptable levels of accuracy fro Favipiravir which is from 505% TO 150%.

Precision

Intra-day was performed analysis of Favipiravir respectively on the same day. Inter-day of the method was checked by repeating analysis of favipiravir on a different day[17] .

LOD And LOQ

The LOD and LOQ were estimated based on 3.3σ/S and 10σ/S criterions respectively, where σ is the standard deviation of the S-intercepts of the regression lines and σ is the slope of the calibration curve[18].

LOD: 0.25ml of standard stock solution was pipetted and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.1ml Favipiravir, were transferred to 10ml volumetric flask and made up with same diluents.

LOQ: 0.25ml of standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.3ml favipiravir, were transferred to 10ml volumetric flasks and made up with same diluents.

Robustness

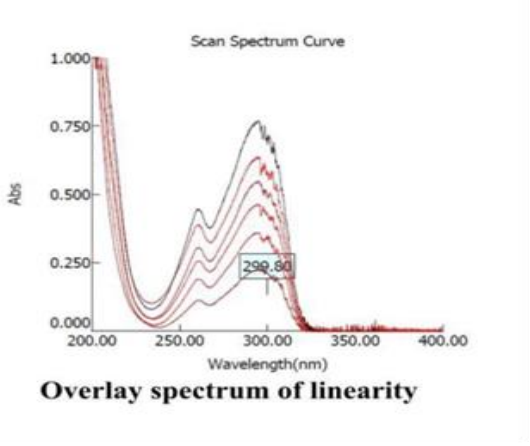
The robustness of an analytical method is a measure of its capacity to remain un effected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The experiment was carried out in different wavelengths.

ASSAY OF THE MARKETED FORMULATION

Marketed tablet formulations of favipiravir were analysed by this method. From the triturate of 20 tablets, an amount equivalent to 10mg of Favipiravir was weighed and transferred to 100ml volumetric flask. The contents of the flask were dissolved in the 50ml of the 0.1N HCL buffer seperately with the aid of ultrasonication for 20min. The solution was filtered and then final volume of the solution was made up to 100ml with same solvents to get a stock solution containing 100µg/mL of Favipiravir in 0.1N HCL. After appropriate dilutions, the absorbance was measured and the concentration of each analyte was determined with the equations obtained from calibration curve.

RESULTS AND DISCUSSIONS

The newly developed method was extensively validated as per international conference on harmonization (ICH) guidelines and parameters. The zero order spectra of Favipiravir were recorded between 200-400nm and the maximum wavelength of Favipiravir was found to be 299.0nm. The overlain spectra were shown.



Overlay spectrum of linearity

Linearity:

Linearity of Favipiravir was assessed by plotting calibration curve of the absorbance versus the concentration. The correlation coefficient (r^2) for Favipiravir was found to be 0.9994. The linear regression data for the calibration plot were indicative of a good relationship between the peak area and concentration over wide range. The results are tabulated in table1 and calibration curve was shown in figure2.

Table 1: Linearity data

Concentration (µg/mL)	Absorbance
0	0
2	0.164
4	0.281
6	0.395
8	0.512
10	0.627
12	0.725

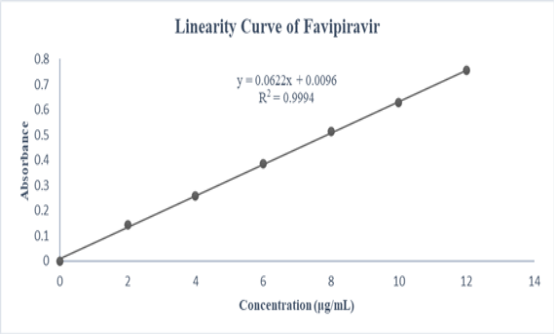


Figure 2: Calibration Curve

Precision:

Precision was determined by intraday and interday of Favipiravir and was expressed as %RSD. The %RSD of intraday precision was 0.567 whereas the %RSD for inter day was 0.263 which are <2. The data was shown in table 2 & 3[19].

Table 2: Intra-Day Precision Data

S. No	Absorbance
1	0.621
2	0.619
3	0.622
4	0.623
5	0.620
6	0.619
AVG	0.621
STDEV	0.002
%RSD	0.263

Table 3: Inter-Day Precision Data

S. No	Absorbance
1	0.621
2	0.628
3	0.631
4	0.626
5	0.628
6	0.630
AVG	0.627
STDEV	0.004
%RSD	0.567

Accuracy:

Standard addition method was employed to determine the accuracy of proposed UV spectrophotometric method. In triplicate, absorbance was recorded and percentage recovery was calculated[20]. In recovery study, the mean recovery was found to be in the range of 100%. The %RSD values were found to be <2, indicates that the method was accurate. The data was represented in table 4.

Table 4: Accuracy data

%LEVEL	Amount Spiked (µg/mL)	Amount Recovered (µg/Ml)	%Recovery	Mean Recovery
50%	8	8.134	101.67	100%
	8	8.102	101.27	
	8	8.118	101.47	
100%	10	9.906	99.60	
	10	9.912	99.12	
	10	10.024	100.24	
150%	12	11.820	98.50	
	12	11.916	99.30	
	12	11.947	99.56	

Robustness:

The methods robustness was established by introducing minor and purposeful modifications to the experimental parameters. The data obtained for each case were evaluated by calculating the % RSD and percentage of the recovery. (ref). Robustness of the method was determined by carrying out the analysis under different wavelength conditions that is 296nm and 299nm by observing the 10ppm concentration solution. %RSD of the above conditions is calculated and data was represented. The data was depicted in table 5.

Table 5: Robustness Data

Condition	Sr.no	Parameter	Absorbance	%Amount	SD	%RSD
Change in wavelength	1	295	0.615	98.17%	0.003	0.49
	2	295	0.621	98.91%	0.0051	0.11
	3	295	0.619			
	1	298	0.617	98.33%	0.002	0.24

Change in wavelength	2	298	0.619			
	3	298	0.620			

ASSAY RESULTS OF MARKETED FORMULATION:

Assay of the marketed formulation was carried out standard solution and sample solutions were observed separately into the system and Absorbance were recorded and drug present in sample was calculated using before mentioned formula. In accordance with ICH guidelines the assay values for the formulation were found to be 99.44%.

SUMMARY TABLE:

Parameters	Favipiravir	Limit
Linearity: Range (µg/ml)	2-12µg/ml	R<1
Regression coefficient	0.9999	
Slope (m)	0.0625x	
Intercept	0.001	
Regression equation (Y=mx=c)	Y=0.0625x+0.001	
Assay (%mean assay)	99.44%	90-110%
Specificity	Specific	No interference of any peak
System precision %RSD	0.567	NMT 2.0%
Method precision %RSD	0.263	NMT 2.0%
Accuracy % recovery	100.08%	98-102%
LOD	0.025	NMT 3
LOQ	0.075	NMT 10
Wavelength minus	0.49	%RSD NMT 2.0
Wavelength plus	0.24	
Ruggedness	0.34	%RSD NMT 2.0

CONCLUSION

Spectrophotometric methods generally do not require complex operations and procedures. It takes less time and is economical. These cases are advantages of the spectrophotometric method over the liquid chromatographic method. The developed methods were simple, rapid, precise, accurate, and easy to apply for routine analysis in the laboratory. The developed procedures were in good agreement with each other and with the reported method.

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